

Subtilisin Carlsberg in Organic Solvents: Binding Conformations of Two Active Site Inhibitors

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Background and objective: It has been shown that exposure of several enzymes to organic media for prolonged periods of time reduce their initial activity, an effect that is particularly detrimental for synthetic applications. The goal of this project was to help, through a theoretical approach, the interpretation of data obtained from Fluorescence and Electron paramagnetic resonance (EPR) studies aimed at resolving the low stability issue of enzymes in organic solvents.

Methods: To explain the experimental results obtained with subtilisin Carlsberg we used a theoretical approach to study the possible binding conformation of a bound fluorophore and of an active site spin label, determining the solvent accessible area of both inhibitors. To accomplish this, we used the software Macromodel in the interface Maestro to do the conformational search and other related calculations.

Results: Our study indicates that two different binding conformations are possible for both the bound unnatural fluorophore and spin label. This is in accordance to the experimental results.

Conclusion: It is possible that prolonged exposure of our model enzyme to organic media induces a change in the polarity of the active site, forcing substrates to adopt a different and less productive binding conformation.

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